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| EXAMINER | |
|---|---|
| TON, THAIAN N | |
| **ART UNIT** | **PAPER NUMBER** |
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| SHORTENED STATUTORY PERIOD OF RESPONSE | MAIL DATE | DELIVERY MODE |
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**Please find below and/or attached an Office communication concerning this application or proceeding.**

If NO period for reply is specified above, the maximum statutory period will apply and will expire 6 MONTHS from the mailing date of this communication.

| Office Action Summary | Application No. | Applicant(s) | |
|---|---|---|---|
| | 09/980,484 | HATZFELD ET AL. | |
| | Examiner | Art Unit | |
| | Thaian N. Ton | 1632 | |

*-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --*

**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

1)☒ Responsive to communication(s) filed on *15 December 2006*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims**

4)☒ Claim(s) *1,8,9,11 and 21-37* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1, 8, 9, 11, 21-37* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

**Application Papers**

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

**Priority under 35 U.S.C. § 119**

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

**Attachment(s)**

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

## DETAILED ACTION

Applicants' Remarks and Amendments, filed 12/15/06, have been entered. Claims 1, 8, 9, 11, 21, 23 are amended; claims 27-37 are newly added; claims 1, 8, 9, 11, 21-37 are pending and under current examination.

The Hatzfeld Declaration, filed concurrently with Applicants' Response, has been considered.

### *Claim Objections*

Claim 21 stands objected to because of the following informalities:

1) Line 10 of the claim recites "activin", this appears to be a misspelling of "antivin". The Thisse reference that Applicants have provided as evidence recites that this compound is antivin. The Hatzfeld Declaration recites the compound as activin. Thus, it is unclear what the spelling of this term is. Appropriate correction or explanation is requested.

### *Claim Rejections - 35 USC § 112*

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 21-26 and newly added claims 28-37 stand rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for:

Methods of maintaining a non-differentiated state of human hematopoietic stem cells, while allowing cell division of said cells, the method comprising administering to said stem cells, TGFβ in the amount of 0.01 pg/ml to 1 mg/ml, in sequential combination with an anti-TGFβ in the amount of 0.1 μg/ml to 10mg/ml,

wherein the stem cells are present in a cell concentration of about 1 to about $10^{10}$ cells per ml.

The specification does not reasonably provide enablement for the breadth of the claims, which encompass using activin; and using stem cells other than hematopoietic stem cells in the claimed methods. This rejection is maintained for reasons of record, advanced in the prior Office action, mailed 6/15/06.

The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

Enablement is considered in view of the Wands factors (MPEP 2164.01(A)). These include: nature of the invention, breadth of the claims, guidance of the specification, the existence of working examples, state of the art, predictability of the art and the amount of experimentation necessary. All of the Wands factors have been considered with regard to the instant claims, with the most relevant factors discussed below.

Applicants' Arguments. Applicants argue that the present specification discloses conditions which enable the claimed invention, and that the Hatzfeld Declaration further demonstrates the use of anti- TGFβ in sequential combination with TGFβ or activin, allows for the multiplication of human stem cells, and particularly embryonic stem cells, while maintaining them in an undifferentiated state. Applicants argue that Thisse *et al.* (cited previously) show that one of skill in the art would have known that activin is a member to the TGFβ family and that none of the publications that were cited in the previous Office actions cast doubt as to whether the claimed method is enabled, because Thomson (cited previously) focus on the effects of LIF on ES cells, and Xi *et al.* compare the mechanisms of platelet factor 4 and TGFβ on progenitor cells, and that both of these factors inhibit progenitor cells, thus, the Xi *et al.* article is not commensurate in scope with the claimed invention. See pages 11-12 of the Response. Applicants argue that claim

33 is now directed to what was expressly identified as being enabled in the prior Office action (p. 3); although, the claim states that ES cells can also be used in this method. Applicants argue that in view of the specification and Hatzfeld Declaration, the claimed method can be used with ES cells. See pages 10-12 of the Response.

Response to Arguments. Applicants' amendments and arguments have been fully considered, but are not persuasive.

The claims are not enabled for their breadth, because they encompass using inhibitors such as TGFβ or antivin, and utilizing the methods with either hematopoietic stem cells (HSCs), embryonic stem (ES) cells, or other stem cells As stated in the Office action, mailed 1/25/05, pages 9-10, the state of the art of culturing stem cells in an undifferentiated state, but allowing them to divide, is found to be unpredictable. ES cells require different factors to maintain them in an undifferentiated state. Furthermore, although the specification and the art support using TGFβ/anti-TGFβ for HSCs, one could not readily apply these methods for ES cells. The specification provides no working examples, guidance or nexus for using the claimed methods for ES cells. Furthermore, the claims recite using TGFβ or activin as the inhibitor. The specification fails to provide guidance with regard to utilizing activin as an inhibitor for either HSCs or ES cells.

Hatzfeld Declaration. The Declaration teaches the culture of human ES cells on human matrices in a serum free medium, SBX. It states that when TGFβ was added to the medium, the stem cells maintained their primitive state, but that when the cells are grown in SBX medium without TGFβ for or activin, the cells undergo differentiation. The Declaration teaches that noggin (100 ng/ml) is used as the anti-TGFβ compound and that the cells treated with cells treated with TGFβ, activin and noggin have enhanced primitivity. See page 3 of the Declaration. The Declaration further teaches the analysis of differentiation gene expression of the ES cells, using Activin A and Noggin (see p. 6, Figure 3) and state the following:

> Human embryonic stem cells in presence of activin (30 ng/ml) divide faster than the in the control conditions or than in the presence of activin and noggin, but express all the tested differentiation markers.
>
> Thus, self-renewal is not achieved when activin is used alone. This stands in contrast to the results obtained when noggin and activin are added in the SBX medium. Indeed, the addition of the anti-inhibitor of cell proliferation (noggin), allows the cells to divide while maintaining their undifferentiated state.

Thus, the Declaration teaches the following:

1. When human ES cells are cultured under specific conditions, particularly, with human matrices and a specific medium (SBX) and treated with TGFβ or activin, the ES cells maintained an undifferentiated state.

2. When the ES cells are treated with noggin (the anti- TGFβ compound) there is an increase in expression of SSEA3, a marker of embryonic stem cell primitivity.

3. When human ES cells are treated with activin alone, they divide and differentiate, but when the cells are treated with both activin and noggin, they maintain an undifferentiated state.

The Declaration is not persuasive for the following reasons:

1. MPEP § 2164.05 states that, "To overcome a prima facie case of lack of enablement, applicant must demonstrate by argument and/or evidence that the disclosure, as filed, would have enabled the claimed invention for one skilled in the art at the time of filing. This does not preclude applicant from providing a declaration after the filing date which demonstrates that the claimed invention works. However, the examiner should carefully compare the steps, materials, and conditions used in the experiments of the declaration with those disclosed in the application to make sure that they are commensurate in scope; i.e., that the experiments used the guidance in the specification as filed and what was well

known to one of skill in the art. Such a showing also must be commensurate with the scope of the claimed invention, i.e., must bear a reasonable correlation to the scope of the claimed invention."

The Declaration is not commensurate in scope with what is disclosed in the application as filed. In particular, the anti TGFβ compound used (noggin) is not contemplated nor disclosed in the as-filed disclosure, with regard to the claimed methods. Thus, the specification does not provide sufficient teachings or guidance to carry out the claimed methods using human ES cells with activin in addition to noggin. Furthermore, the human ES cells are cultured in a specific environment (with human matrices and SBX medium). Neither the claims, nor the as-filed disclosure, provide specific guidance with regard to these culture conditions to carry out the claimed methods utilizing human ES cells.

2. Certain claims are directed to using the claimed methods with either hematopoietic stem cells, or ES cells (claim 21 and its dependent claims, for example), and newly added claims are directed to utilizing the claimed methods on a variety of stem cells (see claims 28 and its dependent claims). The Declaration fails to overcome the prior rejection of record, with regard to utilizing the claimed methods with ES cells, because the Declaration does not teach what is commensurate in scope with the originally filed disclosure. Furthermore, the Declaration does not address utilizing the claimed methods with any other type of stem cell, as is now claimed (for example, skin, liver, pancreas, heart, kidney, bone or neural stem cells). Although the Declaration states that one of skill in the art could practice the claimed invention with activin and other stem cells (see p. 1, last ¶ of the Declaration), there is no support in the Declaration for use of the claimed methods to maintain a non-differentiated state of any of the stem cells contemplated, other than hematopoietic stem cells.

Accordingly, the Declaration fails to overcome the enablement rejection with regard to utilizing embryonic stem cells for the claimed invention, because it

provides experiments that are not commensurate in scope with the claimed invention, using materials and protocol that are neither contemplated nor disclosed in the as-filed disclosure.

Newly added claims. Applicants have now added claims which encompass a broad variety of stem cells (see claim 28 and its dependent claims, for example). The Examiner notes that the breadth of stem cells that could be used in the claimed invention was addressed in the Office action, mailed 1/25/05 (see pages 7-12) and 9/21/05 (see pages 4-7). In particular, it is reiterated that maintaining any stem cell in an undifferentiated state would require undue experimentation by one of ordinary skill in the art. Particularly, the Examiner showed that primate ES cells require specific conditions to maintain them in an undifferentiated state, conditions which are different for other stem cells, such as megakaryocyte progenitor cells in CD34+ cells (see page 9-10 of the Office action, mailed 1/25/05). Furthermore, as stated in the Office action mailed 9/21/05, the culturing of primitive keratinocytes require very low concentrations of a particular inhibitor in order to show an induction of expansion.

Furthermore, the NIH (Stem Cells: Scientific Progress and Future Research Directions, Chapter 4: The Adult Stem Cell, pages 23-42, Department of Health and Human Services, June 2001) is post-filing art that shows that adult stem cells (such as those encompassed by Applicants' amended claims) are unpredictable with regard to their isolation and culture conditions *in vitro*. Each of the recited somatic stem cells has a different origin, different methods to isolate and culture, and differentiation capacity; thus, it is not predictable the one of skill in the art could use the culture techniques contemplated by the instant specification for all of the recited stem cells. The NIH states that, "[A]dult stem cells are dispersed in tissues throughout the mature animal and behave very differently depending on their local environment." See p. 23, 2nd col., 1st ¶. They further state that, "Unlike embryonic stem cells, which are defined by their origin (the inner cell mass of a blastocyst),

adult stem cells share no definitive means of characterization. In fact, no one knows the origin of adult stem cells in any mature tissue." See p. 23, 2nd col., 2nd ¶. They further state that, "To be able to claim that adult stem cells demonstrate plasticity, it is important to show that a cell population exists in the starting tissue that has the identifying features of stem cells. Then, it is necessary to show that adult stem cells give rise to cell types that normally occur in a different tissue. Neither of these criteria is easily met. Simply proving the existence of an adult stem cell population in a differentiated tissue is a laborious process. It requires that the candidate stem cells are shown to be self-renewing and that they can give rise to the differentiated cell types that are characteristic of that tissue." See p. 26, 1st col., Approaches for Demonstrating Adult Stem Cell Plasticity. Applicants provide no specific guidance with regard to the various stem cells (skin, heart, kidney, bone and neural) that are instantly claimed. It is reiterated that in order to practice the claimed invention, one of skill in the art would have to isolate a particular stem cell, of those recited in the claims, determine if TGFβ or activin would result in the inhibition of differentiation, but allow for cell division, and further, determine the amount of these factors to provide sufficient inhibition of differentiation. Applicants have provide the Hatzfeld Declaration in an attempt to show that their claimed method would work on ES cells. However, the Hatzfeld Declaration does not provide guidance for utilizing the claimed method on ES cells, and is silent with regard to applying the claimed method for any other somatic stem cell. One of skill would not be able to rely up upon the state of the art, with regard to the isolation, characterization and maintenance of somatic/adult stem cells, because, as shown by the art of record, as well as the cited NIH article, the conditions required to isolate, characterize and culture adult stem cells is not found to be predictable. Thus, specific guidance, which is not provided by the as-filed disclosure, is required to enable the claimed invention. It is thus maintained that the claimed invention is not enabled for the breadth of stem cells that are

encompassed by the claims, and the scope of enablement is proper, with regard to utilizing Applicants' method with human hematopoietic stem cells.

Thus, when taken with the lack of any particular and specific guidance provided by the specification for with regard to using activin in the claimed methods, as an inhibitor of cell development, and the lack of guidance or teachings provided by the specification with regard to utilizing the claimed methods with ES cells, or any other recited somatic stem cells, as well as the state of the art, which finds maintaining cells in an undifferentiated state to be unpredictable, the lack of nexus between the claimed using the claimed methods with HSCs versus other stem cells, it would have required undue and unpredictable experimentation for one of skill in the art to practice the claimed invention.

*New Matter*

Claims 21-26 and newly added claims 28-32 stand rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. This is a new matter rejection.

*Applicants' Arguments.* Applicants argue that the term "activin" is not new matter, because the term is clearly contemplated within the scope of the invention as inhibitor of cell development, namely because it complies with all of the definitions of an inhibitor of cell development according to the specification (p. 3, lines 10-29, and pages 12-13 of the Response).

Applicants argue that Thisse (provided previously) teach that activin is known as a cytokine member of the TGFβ family, and because the specification teaches that cytokines can be inhibitors of cell development, the recitation of activin

as a inhibitor of cell development does not introduce new matter into the as-filed disclosure. See p. 13, 1st ¶ of the Response.

Applicants provide Kim *et al.* to show that activin is known to be an inhibitor of cell proliferation and cell growth and apoptosis, and Burdette *et al.* teach that activin maintains or enhances the expression of the inhibitors of cyclin-dependent kinases. Accordingly, Applicants maintain that one of skill in the art would have recognized that activin was within the scope of the invention at the time the claimed invention was filed. See page 13 of the Response.

*Response To Arguments.* These arguments have been fully considered, but are not persuasive. It is reiterated that Thisse *et al.* is not within the scope of the instant invention, as their studies are directed to *in vivo* induction of the zebrafish antivin in development of zebrafish embryos. Thisse *et al.* show that overexpression of antivin abolishes mesoderm induction. However, they are not within the scope of the claims, which is *in vitro* assay using human stem cells. Thus, Thisse provide no guidance with regard to antivin being an inhibitor of cell development, with regard to any of the stem cells that are contemplated by the claims. The only nexus that appear to suggest from Thisse *et al.* is that activin is a member of the TGFβ superfamily.

Kim *et al.* has been considered, however, this is post-filing art that is not found to be persuasive. In particular, Kim's teachings are directed to whether activin regulates the cell proliferation of a human gastric cancer cell line. See *Abstract.* However, the claims recite utilizing activin as an inhibitor of cell development. Thus, Kim is not within the scope of the claimed invention, in that they disclose a property of activin (cell proliferation) that is not contemplated by the as-filed disclosure, and is not part of the claimed invention. Furthermore, Applicants fail to provide a nexus between Kim's results, which are directed to *gastric cancer cells*, and the stem cells of the claimed invention.

Burdette *et al.* has been considered; however, this is post-filing art that is not found to be persuasive. Burdette *et al.* teach that activin mediates cellular proliferation in breast cancer cells (see Abstract), however, the claims recite utilizing activin as a inhibitor of cell development. Thus, Burdette is not within the scope of the claimed invention, in that they disclose a property of activin (cell proliferation) that is not contemplated by the as-filed disclosure. Furthermore, Applicants fail to provide a nexus between Burdette's results, which are directed to *breast cancer cells*, and the stem cells of the claimed invention.

Applicants' recitation of that the specification (page 3, lines 10-29) provides description for the term "activin" is not persuasive. This section of the specification is what amounts to a laundry list of gene products that could be used in the claimed invention, and broadly discuss utilizing cytokines. However, this list does not specifically provide a description that would lead one of skill in the art to identify activin as an inhibitor of cell development that would be used in the claimed invention. At the most, this part of the specification would only direct the skilled artisan to use TGF-β, or a cytokine. Even by arguing that because by virtue of the fact that Thisse *et al.* teach that activin is a member of the TGFβ superfamily, one of skill in the art would not recognize that Applicants had possession of the claimed invention, because the TGFβ superfamily encompasses a large number of members many with variable functions. For example, Attisano *et al* (**Cytokine & Growth Factor Reviews**, 7(4): 327-339, 1996) state that, "Transforming growth factor-β superfamily members exert their diverse biological effects through their interaction with heteromeric receptor complexes of transmembrane serine/threonine kinases ... The composition of these complexes can vary significantly due to the promiscuous nature of the ligands and the receptors, and this diversity of interactions can yield a variety of biological responses." See Abstact. They teach that this family now includes almost 40 members from animals as diverse as pacific oysters, *C. elegans, Drosophilia*, and humans. See p. 327, 1st col. Additionally, Chang *et al.* (**Endocrine**

Rev, 23(6): 787-823, 2002) teach that members of the TGFβ superfamily include TGFβs, growth differentiation factors, bone morphogenetic proteins, activins, inhibins, and glial cell-line derived neurotrophic factors, and that these members have diverse roles in both developmental and physiological pathways (see Abstract). Thus, given that the art recognizes many family members in the TGFβ superfamily, each with different, diverse biological functions, and the as-filed disclosure provides no specific support for utilizing activin in the claimed methods, it is maintained that this recitation introduces new matter into the as-filed disclosure.

MPEP § 2163.02 teaches that, "Whenever the issue arises, the fundamental factual inquiry is whether a claim defines an invention that is clearly conveyed to those skilled in the art at the time the application was filed...If a claim is amended to include subject matter, limitations, or terminology not present in the application as filed, involving a departure from, addition to, or deletion from the disclosure of the application as filed, the examiner should conclude that the claimed subject matter is not described in that application. Applicants' arguments are not persuasive because the specification, as originally filed, does not contemplate or support activin as an inhibitor of cell development of hematopoietic stem cells, embryonic stem cells, or any of the other stem cells that are recited in the claims. The lack of support in the specification for utilizing activin, in the claimed methods, has been determined as new matter.

MPEP § 2163.06 notes, "If new matter is added to the claims, the examiner should reject the claims under 35 U.S.C. 112, first paragraph - written description requirement. In re Rasmussen, 650 F.2d 1212, 211 USPQ 323 (CCPA 1981)." MPEP § 2163.06 further notes, "When an amendment is filed in reply to an objection or rejection based on 35 U.S.C. 112, first paragraph, a study of the entire application is often necessary to determine whether or not "new matter" is involved. Applicant should therefore specifically point out the support for any amendments made to the disclosure."

To the extent that the methods are not described in the instant disclosure, claims 21-26 and newly added claims 28-32 are also rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention, since a disclosure cannot teach one to make or use something that has not been described.

### *Claim Rejections - 35 USC § 112*

The prior rejection of claims 9, 11, 23 under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention is withdrawn in view of Applicants' amendment to the claims.

### *Claim Rejections - 35 USC § 103*

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35

U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1, 8, 9, 11, 21-26 and newly added claims 27-37 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Hatzfeld *et al.* (cited previously) when taken with Fortunel *et al.* (cited previously).

*Applicants' Arguments.* Applicants argue that there are two types of stem cell multiplication: amplification and self-renewal, and in particular, amplification does not prevent or inhibit differentiation of the stem cells, and that the stem cells rapidly lose their properties as they undergo differentiation, whereas in self-renewal, a large amount of undifferentiated stem cells is recovered. Applicants argue that the HPP-Q assay, as discussed by the present specification, and disclosed by both Hatzfeld and Fortunel, was developed to evaluate the differentiation potential of stem cells. In particular, Applicants argue, this assay is a rapid differentiation culture assay used to evaluate the differentiation potential of quiescent high proliferative potential cells after activation with anti-TGF-β which Applicants argue shows that the HPPQ assay is used as a diagnostic test of the differentiation potential of cells, and not with regard to the self-renewal of stem cells. Applicants argue that this assay is further distinguishable from the claimed invention in that the assay utilizes a very short term proliferation of cells that grow as colonies in semi-solid medium, which is not suitable for carrying of the multiplication of stem cells without differentiation, as recited by the claims.

*Response to Arguments.* These arguments have been fully considered, but are not persuasive.

Applicants' argument that the HPPQ assay was disclosed to evaluate "differentiation potential of stem cells" is not persuasive. MPEP §2105 states that, "The reason or motivation to modify the reference may often suggest what the inventor has done, but for a different purpose or to solve a different problem. It is not necessary that the prior art suggest the combination to achieve the same

advantage or result discovered by applicant." The specification teaches that the HPP-Q test, "allows for the verification that the inhibitor of cell development, in particular, TGF-β, is maintaining the stem cells, in particular hematopoietic stem cells, at rest." See p. 12, lines 18-20. Thus, the as-filed specification clearly teaches that this assay is used to evaluate maintenance of HSCs at rest. This is clearly supported by Hatzfeld *et al.*, who state that this test can be used for the expansion of HSCs (see Abstract, last ¶) and by Fortunel *et al.*, who teach utilizing the HPPQ test to identify HPPQ cells as, "primitive progenitors for which very low concentrations of TGF-β1 can down-modulate receptors controlling their cycling status. When a quiescent stem/progenitor cell is activates, it maintains for a few division its immature phenotype and its high proliferative potential. ... Similarly, we have evidence that even after activation, HPP-Q cells maintain for at least one division their ability to return to the quiescent state in response to physiological concentrations of TGF-b1." See p. 1872, 2nd col., 1st ¶. Thus, both Hatzfeld and Fortunel teach that the HPPQ assay is designed to test for cells that are able to divide but also maintain their primitive stem cell status.

With regard to Applicants' arguments that the HPPQ assay is further distinguishable from the claimed invention in that the assay utilizes a very short term proliferation of cells that grow as colonies in semi-solid medium, which is not suitable for carrying of the multiplication of stem cells without differentiation, as recited by the claims, it is noted that Applicants are arguing limitations that are not found within the claims. The claims simply require maintaining a non-differentiated state of HSCs, while allowing for cell division of the cells, by allowing cells to divide until they reach a particular, pre-determined number (see claim 1, lines 8-9). Claim 21 does not even require the production of a particular number of cells, and dependent claims merely recite the production of an unclaimed number of cells. Similarly, claims 28 and 33 only recites the production of a "predetermined number" of cells (lines 8-9). Thus, because Hatzfeld teach that one could use this

assay with either non-purified progenitors in semi-solid culture, or highly purified progenitors in long-term liquid culture to characterize and phenotype the stem cell compartment, and Hatzfel disclose the same, the Examiner maintains that the combined teachings of Hatzfeld and Fortunel provide sufficient guidance and motivation to arrive the claimed invention, with a reasonable expectation of success.

With regard to Applicants' arguments that Hatzfeld teach "transient activation", which includes differentiation but not self-renewal, and that they do not teach a way to keep proliferating cells in an undifferentiated state, nor the use of anti-TGF-b in a sequential manner with TGF-β or activitin to avoid the differentiation of cells, it is maintained that Hatzfeld teach that the their HPPQ assay renders quiescent HSCs responsive to cytokines which can improve the expansion of the cells. See last ¶. They further suggest that using their HPPQ assays, either non-purified or purified progenitor cells can be cultured, for either short term or long-term use, such as clinical expansion of the cells. Accordingly, this rejection is maintained.

Hatzfeld teach that endogenous or added TGF-β down-modulates various cytokine receptors, and that this effect can be suppressed within 6 hours by the addition of anti-TGF-β antibodies, or antisense nucleotides. Hatzfeld study the release from TGF-β growth inhibition of high proliferative potential-quiescent primitive progenitors to understand whether this inhibitor is a central regulator of the stem cell compartment. They teach that these observations are used in developing an *in vitro* assay which combines receptor induction by anti-TGF-β together with optimal cytokine stimulation which can be performed using non purified hematopoietic progenitors. They teach that this method can render quiescent primitive progenitors responsive to optimal combinations of cytokines to improve the *in vitro* expansion of clinical samples. They teach the neutralization of an inhibitor of cell development (i.e., TGF-β). They further teach using these methods on CD34+ cells.

Hatzfeld *et al.* differ from the claimed invention, in that they do not teach specific amounts of added TGF-β or specific amounts of anti-TGFβ. However, prior to the time of the claimed invention, Fortunel *et al.* teach culturing CD34+ stem cells with 2 or 5 ng/ml of TGFβ and 5μg/ml of anti-TGFβ blocking antibody (see page 1868, col. 1-2, Hematopoietic growth factors and antibody). They teach that anti-TGF β releases the cells from quiescence, thus allowing them to divide (see Abstract). They teach amounts of TGFβ, which are within the range required by claims. They teach culturing cells, which falls within the claimed range of 1 to about $10^{10}$ cells per ml, as required by the claims.

Thus, given the combined teachings of Hatzfeld *et al.* and Fortunel *et al.*, it would be obvious for one of skill in the art to use the specific ranges, as taught by Fortunel *et al.*, to the assay, as taught by Hatzfeld *et al.*, with a reasonable expectation of success. One of ordinary skill would have been motivated use the specific ranges, as taught by Fortunel they show that the anti-TGFβ blocking antibody effectively neutralizes exogenously added TGFβ, (p. 1868, 2nd col., 1st ¶), and they suggest using TGFβ/ anti-TGFβ in an assay detect quiescient progenitor cells (HPP-Q cells) (p. 1870-71, bridging ¶), the same assay suggested by Hatzfeld *et al.*.

Thus, the claimed invention, as a whole, is clearly *prima facie* obvious in the absence of evidence to the contrary.

*Conclusion*

No claim is allowed.

THIS ACTION IS MADE FINAL. Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the Examiner should be directed to Thaian N. Ton whose telephone number is (571) 272-0736. The Examiner can normally be reached on Monday through Thursday from 7:00 to 5:00 (Eastern Standard Time). Should the Examiner be unavailable, inquiries should be directed to Peter Paras, SPE of Art Unit 1632, at (571) 272-4517. Papers related to this application may be submitted to Group 1600 by facsimile transmission. Papers should be faxed to Group 1600 via the Official Fax at (571) 273-8300. The faxing of such papers must conform with the notice published in the Official Gazette, 1096 OG 30 (November 15, 1989).

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